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TOXIC AND ANTAGONISTIC EFFECTS OF SALTS ON WINE YEAST (*SACCHAROMYCES ELLIPSOIDEUS*)

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Introduction

Most of the published studies of wine yeast deal with its activities as related to wine making. Its botanical characteristics and still less its fundamental physiological reactions have apparently received little attention. Among the most important and interesting investigations of higher plants, bacteria and animals in recent years have been studies of the effects of various single salts and various combinations of salts on the physiology of these organisms. For example, it has been found by Osterhout[6, 7] that practically all of the simple salts, such as sodium chloride, potassium chloride, calcium chloride, etc., have a decided toxic action upon the plant when it is subjected to the action of a single pure salt. Further, if certain combinations of two or more salts were used in certain ratios the toxicity was reduced. This reduction of toxicity is commonly termed antagonism between the salts used. It was found also that a combination of all the salts in the ratios in which they occur in the soil solutions or in other solutions to which the plant is accustomed afford the best conditions for growth. Such a combination is spoken of as a physiologically balanced solution.

Loeb working with marine animals and C. B. Lipman with soil and other bacteria obtained results similar to those obtained by Osterhout with the higher plants. As was to be expected, the reaction of animals to the salts was not identical with that of bacteria, nor does either reaction follow the behavior of the higher plants closely. In general, however, the results with all three classes were of the same kind; that is, single salts, hitherto considered non-toxic, were found to be toxic to organisms, while various combinations of these salts showed antagonism or reduction of toxicity in the presence of each other. In accordance with these facts, a physiologically balanced solution can be made by using proper concentrations and proportions of the various salts found in the solutions to which the organism is accustomed.

So far as the writer could ascertain, no one has investigated the behavior of yeast in this respect. Results of investigations of the effects of the heavy metallic salts, such as mercuric chloride, silver nitrate, etc., on yeast have been published (Bokarny[12]), but nothing has appeared upon the toxic and antagonistic effects of such salts as sodium chloride, potassium chloride, calcium chloride, and magnesium chloride. This field therefore seemed especially inviting, and it was with the idea of studying the fundamental relations existing between yeast and the chlorides that the writer undertook the work summarized in the following pages.

Acknowledgments

The experiments on which this paper is based were carried out under the general supervision of Professor W. V. Cruess, and I am indebted to Professor F. T. Bioletti for suggestions and critical reading of the manuscript.

Method of Experimentation

In the selection of a yeast for my investigation I was led to use the wine yeast, *Saccharomyces ellipsoideus,* by the fact that it is one of the most useful of all yeasts and is universally used in wine making. It is also one of the most vigorous, is easy to grow, and gives definite results in a few days.

The particular yeast, no. 66, used in this experiment, was isolated by William V. Cruess from one of the wineries in northern California. It has been found by repeated trials that specimens of *Saccharomyces ellipsoideus* collected from different sources are not identical as to their physiological characters in every respect, and so do not respond in different salt solutions in the same way. An experiment showing this will be described later.

Although work on this line may not have immediate practical value to the zymologist, it is of considerable scientific interest. It is with this thought that these experiments have been carried out in the Laboratory of Zymology.*

The salts tested in these experiments were the chlorides of potassium, magnesium, calcium and sodium, each being taken up separately. The reason for choosing these chlorides was that their metallic ions (cations) are those most abundant in the ash of grape juice. Besides, Loeb[1] and Lipman[11] have shown that the positive ions of these salts have the most effect, while their negative ions (anions) have the least. Owing to the fact that the effect of the chlorine ion is uniform in all cases, the metallic ions show their characteristic effects on the yeast culture very clearly.

Choice of Solution.—It has been shown by Loeb with marine animals (*Fundulus*), by Osterhout with higher plants (wheat), and by Lipman with soil bacteria (*Bacillus subtilis*) that, for the growth of living organisms, a nutrient solution must be physiologically balanced. In order to grow the yeast in a medium whose constituents were known both in quality and quantity, it was necessary to prepare a nutrient solution from pure materials. Such a solution must contain an adequate amount of nitrogen and phosphorus in order that the yeast may grow rapidly. For this purpose a number of substances were tried, such as Witte's peptone, asparagin, urea, and ammonium phosphate, in different concentrations, with pure cane sugar or pure dextrose. Witte's peptone proved to be impure, being very high in ash, and the others did not give satisfactory results. As dextrose is not easily available in the market at present, pure cane sugar had to be used as a carbohydrate food and as a source of fermentable material.

Later a synthetic solution was made with hydrolized pure cane sugar, phosphoric acid, and ammonia, which was suitable for the growth of yeast for experimental purposes. Although this synthetic solution produces a slower rate of growth than grape juice, which is a perfect physiologically balanced solution for yeast fermentation, it gives sufficient growth for experimental work. To make it, a 50 per cent pure cane sugar syrup was made with distilled water and a measured amount (1 gram per 100 c.c. of syrup) of phosphoric acid added. This syrup was hydrolized by boiling for one-half hour on a slow fire, and was then neutralized with dilute ammonium hydroxide. Litmus solution was used to test the neutrality of the syrup. The

* These experiments were carried out under the general supervision of William V. Cruess.

ammonium phosphate that eventually formed furnished both the nitrogen and phosphorus needed by the yeast. As yeast cells grow easily in moderately acid, but not in alkaline solutions, the syrup was left slightly acid, being tested by titration with N/10 solution of sodium hydroxide. To facilitate the work, the syrup was boiled down to 65°Balling and put into a corked bottle. From this concentrated synthetic solution a measured quantity was drawn off and diluted with distilled water to 5°Bal. for use in the cultures.

Method of Determining the Activity of the Yeast.—The experiments were carried on in a series of 200 c.c. Erlenmyer flasks. To each flask the weighed amount of salt was added and 100 c.c. of the diluted synthetic solution placed in the flask by means of a 100 c.c. pipette.

The salts were weighed according to their respective molecular concentrations. The flasks as soon as filled were plugged with cotton and sterilized. After they had cooled down to the room temperature they were inoculated with the yeast from a new culture. For this purpose the new culture was prepared in a 200 c.c. Erlenmyer flask containing 100 c.c. of the synthetic solution. The yeast thus became habituated to this solution and therefore grew rapidly and uniformly in the flasks. The new culture was transferred from a mother culture in grape juice and put into the incubator for forty-eight hours at 28°C. At the end of this period the flasks containing the salts were inoculated with one cubic centimeter of the new culture. After inoculation, the flasks were put into the incubator, which was kept at an approximately even temperature of 28°C. during the entire experiment.

As the alcoholic fermentation in the synthetic solution was not rapid enough to serve as a criterion, the multiplication of the cells was taken as the measure of the activity of the yeast. Accordingly a microscopical count was made every forty-eight hours with a calibrated microscope. Five counts were made in each experiment, during a period of about twelve days. In every case two blanks, with no added salts, were made up, and the tables given represent the average of two sets of duplicate experiments, except in the cases of potassium chloride and magnesium chloride, where the results were so close that only the first set of duplicates was used. It may be added that the incubator did not keep exactly the same temperature throughout the experiments, but ranged from 27°C. to 29°C. This difference of 2°C., however, did not interfere appreciably with the uniformity of growth and the check flasks controlled any slight variation it may have caused.

Salts Used.—During February, 1916, a series of experiments in two parts was planned. The first concerned the toxicity of the single salts and the second the antagonistic effects of all their binary and ternary combinations, as follows:

I. Toxicity of the Single Salts

1. KCl
2. $MgCl_2$
3. $CaCl_2$
4. NaCl

II. Antagonistic Effects of Combinations

A. Binary Combinations

1. $MgCl_2 + CaCl_2$
2. $KCl + CaCl_2$
3. $MgCl_2 + NaCl$
4. $KCl + NaCl$
5. $KCl + MgCl_2$
6. $CaCl_2 + NaCl$

B. Ternary Combinations

1. $NaCl + KCl + CaCl_2$
2. $NaCl + KCl + MgCl_2$
3. $NaCl + MgCl_2 + CaCl_2$
4. $KCl + CaCl_2 + MgCl_2$

A. Experiments With Single Salts—Toxic Effects

In all cases chemically pure salts (Baker's analyzed) were used. Each amount of the single salts was carefully weighed and put into the flasks, except .001M and .01M, which were added as solutions of known strength according to molecular weights. The following proportions were taken:

1. KCl — .001M to 2.2M* Molecular
2. $MgCl_2$ — .001M to 1.2M
3. $CaCl_2$ — .001M to .7M
4. NaCl — .001M to .2M

All of these solutions were clear except the calcium chloride, which gave an appreciable amount of coagulated precipitate of calcium phosphate with the phosphorus of the synthetic solution. This, however, did not interfere with the experiment, as the precipitate disappeared with the growth of the yeast and the solution finally became almost clear. The tables and curves given in each case show the growth of yeast at every forty-eight hours in the different molecular concen-

* M represents the degree of concentration in a solution which contains one gram molecule of the substance in one litre of solution.

trations of each salt as indicated above. In the microscopical count one million yeast cells per cubic centimeter was taken as an appreciable number; below that it was not considered that any appreciable growth had taken place.

SERIES I—POTASSIUM CHLORIDE

Thirty 200 c.c. Erlenmyer flasks were arranged in duplicate, including two blanks. The first pair had no salt added and was used as a check. The second pair had .001M KCl and the third .01M KCl, and so on to the last pair, which had 2.2M KCl, as shown in the table below. Then, with a 100 c.c. pipette one hundred cubic centimeters of the diluted synthetic solution (5°Bal.) were put into each flask. The flasks were plugged with cotton, sterilized, and inoculated with yeast, as stated before, and put into the incubator, and counted every forty-eight hours. The results are shown in table 1 and the curves in figure 1.

The curves have been plotted from the results of every forty-eight hours' growth, taking the various concentrations of potassium chloride as abscissae and the number of yeast cells, counted in millions, as ordinates (fig. 1). Following the table and the curves, it is evident at a glance that potassium chloride up to the concentration of .2M accelerates the multiplication of the yeast. Beyond this it becomes gradually more and more toxic until at 2.2M the yeast cells entirely cease to multiply. Both Magowan[10] and Lipman,[11] especially the former, found a strong resemblance between potassium chloride and sodium chloride in their action on wheat and on *Bacillus subtilis*. The yeast shows physiological characteristics differing from those of either the bacteria or the wheat.

Lipman[11] found sodium chloride the least toxic to *Bacillus subtilis* and potassium chloride second. Magowan,[10] with wheat, found this position reversed, the potassium chloride being less toxic. Both observers found that these salts were very similar in their degree of toxicity. To yeast sodium chloride is the most toxic of the four salts and potassium chloride the least. Ostwald[4] in experimenting with animals (*Grammarus*) found potassium chloride the most toxic, and Loeb's work[2, 3] with *Fundulus* corroborates this to a certain extent. The reaction of yeast, therefore, differs from that of bacteria of the higher plants or of animals.

TABLE 1—TOXIC EFFECT OF KCL ON *Saccharomyces ellipsoideus*

M.KCl	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
.00	1,695,000	7,237,000	11,636,000	12,892,000	15,835,000
.001	3,325,000	10,786,000	15,400,000	16,217,000	19,670,000
.01	7,797,000	15,600,000	18,905,000	21,479,000	23,568,000
.1	7,262,000	20,558,000	24,208,000	24,608,000	27,915,000
.2	5,650,000	18,493,000	29,689,000	28,783,000	30,541,000
.4	5,425,000	20,227,000	25,006,000	27,560,000	28,914,000
.6	1,130,000	8,535,000	20,928,000	22,802,000	23,802,000
.8	809,000	5,298,000	17,670,000	19,508,000	20,982,000
1.0	226,000	6,787,000	15,205,000	17,986,000	18,453,000
1.2	....	1,102,000	8,986,000	16,207,000	16,951,000
1.4	....	452,000	8,765,000	11,584,000	12,882,000
1.6	....	....	3,204,000	5,794,000	8,232,000
1.8	....	....	904,000	1,243,000	6,252,000
2.0	....	....	....	904,000	2,051,000
2.2	....	....	....	....	226,000

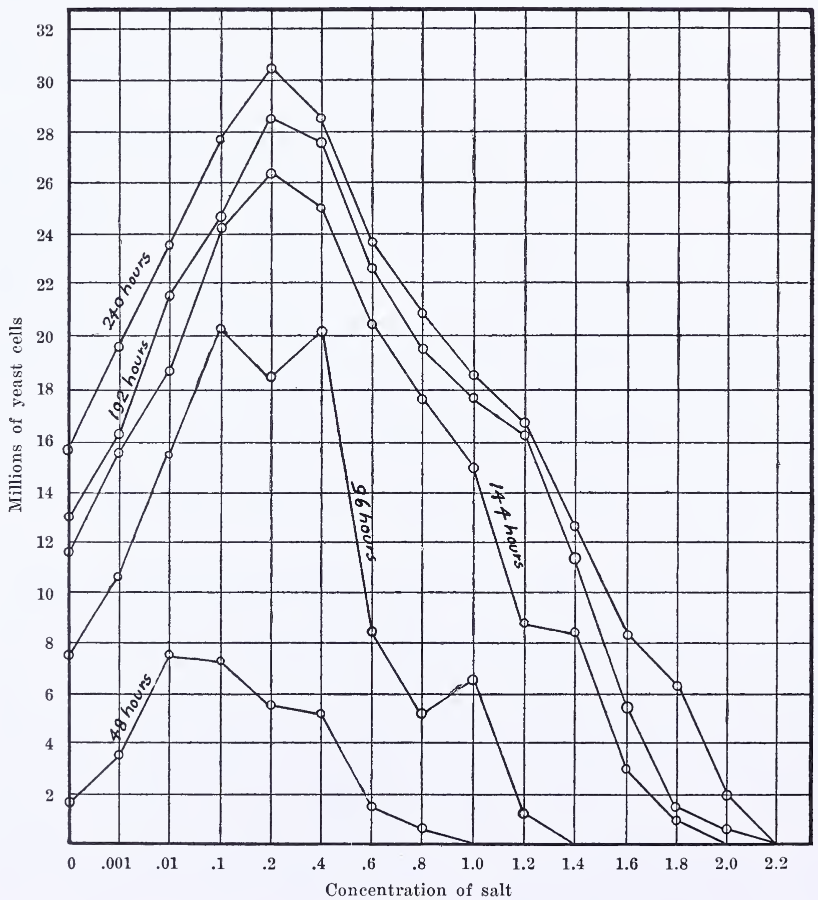

Fig. 1.—The ordinates represent millions of yeast cells and the abscissae, the various concentrations of KCl. The ordinates at 0 represent the number of yeast cells in the check cultures.

SERIES II—MAGNESIUM CHLORIDE

The same method of procedure was used with $MgCl_2$ as with KCl. The results are shown in Table 2.

TABLE 2—TOXIC EFFECT OF $MgCl_2$ ON *S. ellipsoideus*

M. $MgCl_2$	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
.00	2,412,000	8,108,000	12,205,000	16,837,000	17,202,000
.001	4,972,000	10,753,000	13,673,000	18,871,000	19,775,000
.01	8,751,000	15,798,000	18,703,000	19,588,000	20,374,000
.1	3,482,000	12,227,000	20,521,000	25,013,000	26,501,000
.2	2,356,000	7,204,000	11,342,000	15,530,000	18,617,000
.4	1,695,000	5,400,000	9,504,000	12,837,000	17,413,000
.6	989,000	2,157,000	7,332,000	11,253,000	11,905,000
.8	226,000	1,130,000	4,294,000	6,943,000	8,436,000
1.0	....	226,000	1,875,000	2,712,000	2,904,000
1.2	....	....	....	....	226,000

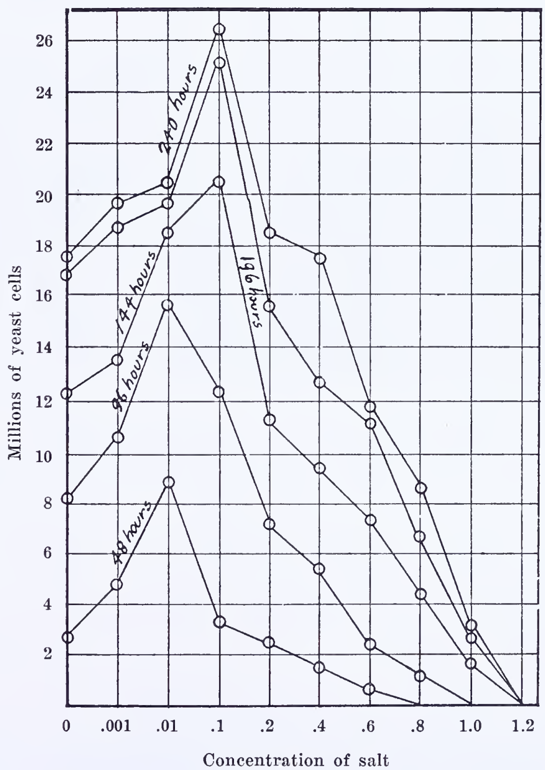

Fig. 2.—The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of magnesium chloride. The ordinate at 0 represents the number of yeast cells in the check cultures.

From both table 2 and the curves in figure 2, it is evident that magnesium chloride is more toxic than potassium chloride. Up to the concentration of .1M, it is favorable to the growth of yeast, but beyond this it becomes more and more toxic until at 1.2M concentration there is little or no growth at all. In the case of yeast, magnesium chloride and calcium chloride show less similarity than that found by Lipman with soil bacteria, and the toxic effect of magnesium chloride is nearer to that of calcium chloride than to that of potassium chloride. Magnesium chloride is not so toxic to yeast as Lipman found it with *Bacillus subtilis*. In the case of yeast, .7M concentration of calcium chloride altogether inhibits its growth. The same concentration, however, of magnesium chloride allows an appreciable number of yeast cells to grow, and the same toxic effect as that of .7M $CaCl_2$ is not attained until a concentration of 1.2M $MgCl_2$ is reached. In fact, magnesium chloride stands midway between the two extremes of toxicity of these four salts, namely, the more toxic NaCl and $CaCl_2$ and the less toxic, KCl.

Loeb,[1, 2] with marine organisms, found that a .5M solution of magnesium chloride inhibits the development of embryos in the eggs of *Fundulus,* and that even .125M $Ca(NO_3)_2$ is toxic. In his experiment with soil bacteria Lipman[11] has met with about the same result. He found that .4M $MgCl_2$ inhibits the growth of *Bacillus subtilis,* while for the same effect on yeast a concentration of 1.0M $MgCl_2$ is needed. But Magowan[10] has shown that with wheat magnesium chloride is the most toxic of all the four salts; in this respect yeast resembles neither the animals, nor bacteria, nor the higher plants.

It must be noted that the magnesium chloride used in all the experiments was $MgCl_2.6H_2O$, as this is less hygroscopic than the same salt having two molecules less of water ($MgCl_2.4H_2O$), which is difficult to weigh accurately. However, magnesium chloride was found more toxic than potassium chloride and more favorable than calcium chloride, which is directly opposite to the result obtained with higher plants.

SERIES III—CALCIUM CHLORIDE

The experiment with calcium chloride was carried on in the same way. From both table 3 and the curves in figure 3, it is evident that .01M concentration of $CaCl_2$ gives the highest growth, while beyond this favorable concentration $CaCl_2$ is more and more toxic. In its toxicity to yeast, $CaCl_2$ stands second, NaCl being the most toxic of the four salts. A .5M concentration of $CaCl_2$ allows an appreciable growth of yeast, while even .2M NaCl stops all growth.

TABLE 3—TOXIC EFFECT OF $CaCl_2$ ON *S. ellipsoideus*

M. $CaCl_2$	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
.00	2,599,000	8,136,000	11,752,000	13,108,000	16,336,000
.001	3,051,000	9,989,000	12,656,000	13,965,000	17,751,000
.01	3,503,000	11,050,000	13,926,000	15,885,000	19,251,000
.1	226,000	6,034,000	8,468,000	10,904,000	16,674,000
.2	....	1,695,000	3,256,000	3,821,000	15,778,000
.3	....	904,000	1,130,000	3,090,000	14,561,000
.4	....	226,000	904,000	1,130,000	9,771,000
.5	....	....	226,000	987,000	2,935,000
.6	....	....	....	226,000	904,000
.7	....	....	....	....	226,000

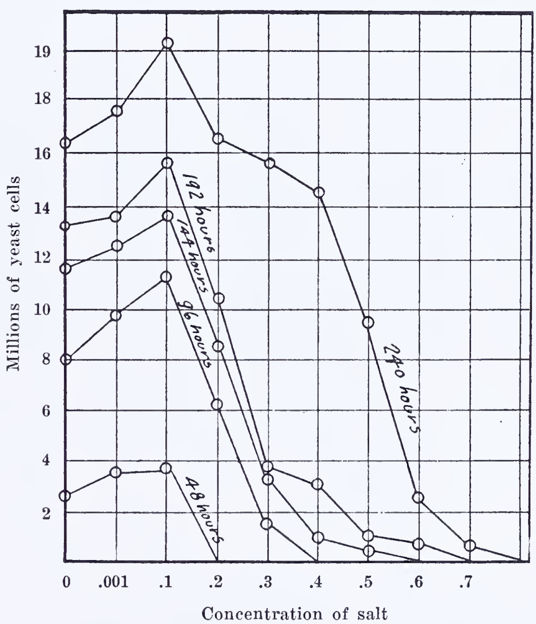

Fig. 3.—The ordinates represent the number of yeast cells in millions and the abscissae the concentration of $CaCl_2$. The ordinate at 0 represents the number of yeast cells in the check cultures.

The work of other investigators with regard to the toxicity of $CaCl_2$ shows a general agreement with the results obtained by Loeb with *Fundulus,*[3] Ostwald[4] with fresh water, *Grammarus,* and Lipman[11] with soil bacteria, all of which show $CaCl_2$ to be extremely toxic. An exception to this general statement is found in the work of

Magowan, who showed in her experiments that, in the case of wheat, $CaCl_2$ is the least toxic of the four salts. Here also we find that yeast exhibits a peculiar physiological character which does not agree with either of the above divisions, the animals or the plants. Perhaps this may throw some light on the relation of yeast to these two groups.

SERIES IV—SODIUM CHLORIDE

As NaCl is the most toxic of all the salts, only a few pairs of flasks were taken, from .001M to .2M concentration, together with a pair of blanks. The experiment was carried on in the same way as the others. From table 4 and the curves in figure 4, it is evident that NaCl is the most toxic to the yeast. Even the concentration of .01M NaCl did not stimulate the growth of yeast, as it did with the other salts. The highest growth in this case was in .001M, and beyond that it was toxic.

TABLE 4—TOXIC EFFECT OF NaCl ON *S. ellipsoideus*

M.NaCl	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
.00	2,424,000	8,059,000	9,267,000	11,978,000	15,142,000
.001	3,819,000	9,605,000	12,430,000	12,995,000	17,967,000
.01	3,164,000	7,458,000	10,283,000	10,504,000	13,060,000
.1	226,000	452,000	452,000	452,000	1,130,000
.2	--------------	--------------	--------------	--------------	226,000

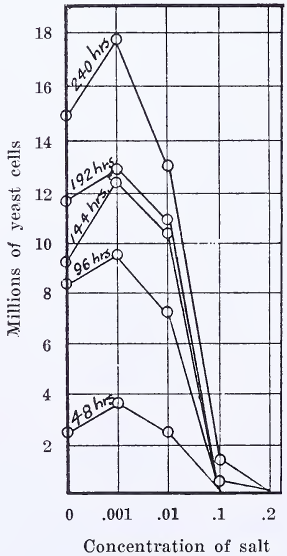

Fig. 4.—The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of NaCl. The ordinate at 0 represents the number of yeast cells in blank cultures.

NaCl shows a directly opposite reaction with yeast from that found by Lipman[11] with soil bacteria. Both Loeb and Ostwald found NaCl to be toxic for animals, but less so than we have found with yeast. The toxicity of NaCl to animals may be compared with the toxicity of $NaCl_2$ to yeast. Loeb[3] found it impossible to develop embryos in the egg of *Fundulus* at .625M NaCl. Osterhout[8, 9] found that a .375M solution of sodium chloride is fairly toxic to marine plants. Young plants of a fresh-water alga, *Vaucheria sessilis,* could not live at a .094M concentration of sodium chloride, and even a concentration of .0001M NaCl was found to be toxic. Magowan has shown that sodium chloride is very toxic to wheat seedlings and down to .02M the root hairs did not grow at all. The relation of yeast to plants is thus to a certain extent shown by similar physiological behavior.

It may be noted here that the experiments with yeasts have been conducted on the same general principle followed by previous investigators with animals, plants, and bacteria. The number of yeast cells was taken as the measure of multiplication or activity and was determined by a microscopical count of each flask every forty-eight hours. It must be admitted that experimental errors may occur in counting, but as the numbers were taken from the average results of two sets of duplicates it does not interfere with the validity of the final result, as the range of variation between the results of these two sets of duplicates was only between 0 and 10 per cent calculated from the mean variation.

SERIES V—EFFECT OF THE TOXICITY OF SALTS ON THE MICROSCOPICAL APPEARANCE OF YEAST CELLS

It is generally known that all salts at certain concentrations are more or less toxic to living organisms. Yeast shows its physiological condition in relation to various salts in characteristic ways. It is evident from the above experiments that in this respect it occupies a place between the animal and the plant kingdoms. Although yeast grows normally in a physiologically balanced solution, for which grape juice answers in every way, the addition of a small amount of a favorable salt, as potassium chloride, may stimulate the growth a great deal. This is of some practical zalue to zymologists.

Yeast is affected very remarkably by the toxicity of salts at different concentrations. In the extreme concentrations it apparently dissolves. This occurs in the cultures having 2.2M KCl, 1.2M, $MgCl_2$, .7M $CaCl_2$, and .2M NaCl respectively. At lower concentrations there is a degenerated condition, various shapes occurring, as shown in figure C. Such diseased cells show a heavy black membrane, especially in the case of $CaCl_2$ and NaCl, with transparent cell-illusions or black spots within the cells. Moreover, they vary in size. This variation in size occurs also with KCl and $MgCl_2$, but in these cases the yeast cells are larger than with $CaCl_2$ and NaCl. In all instances, as the concentration of salt increases beyond the favorable degree of concentration the cells become smaller and smaller until finally, in the extreme concentrations, they dissolve. Table 5 (*a, b, c, d*) and the curves in figure 5 (*a, b*) show the effect on the size of yeast cells in different salt solutions.

TABLE 5*a*—EFFECT OF KCL ON SIZE OF YEAST CELLS (*S. ellipsoideus*)

Concentration of salt (M.KCl)	Av. length and breadth of yeast cells in Mu.	Av. volume yeast cells calculated from length and breadth
.00	4.7 × 4.6	77
.001	5.4 × 5.4	122
.01	6.7 × 6.7	232
.1	6.7 × 6.7	232
.2	6.7 × 6.7	232
.4	6.6 × 6.6	223
.6	6.6 × 6.6	223
.8	5.8 × 5.8	151
1.0	5.8 × 5.8	151
1.2	5.8 × 5.8	151
1.4	5.8 × 5.8	151
1.6	4.9 × 4.9	91
1.8	4.9 × 4.9	91
2.0	3.3 × 3.3	28
2.2	3.3 × 3.3	28

TABLE 5*b*—EFFECT OF $MgCl_2$ ON SIZE OF YEAST CELLS (*S. ellipsoideus*)

Concentration of salt (M.$MgCl_2$)	Av. length and breadth of yeast cells in Mu.	Av. volume yeast cells calculated from length and breadth
.00	4.5 × 4.5	71
.001	5.1 × 5.1	103
.01	6.2 × 6.2	185
.1	6.2 × 6.2	185
.2	5.0 × 5.0	98
.4	5.1 × 5.1	103
.6	5.0 × 5.0	98
.8	4.9 × 4.9	91
1.0	4.4 × 4.4	66
1.2	3.3 × 3.3	28

TABLE 5*c*—EFFECT OF $CaCl_2$ ON SIZE OF YEAST CELLS (*S. ellipsoideus*)

Concentration of salt ($M.CaCl_2$)	Av. length and breadth of yeast cells in Mu.	Av. volume of yeast cells calculated from length and breadth
.00	4.7 × 4.4	70
.001	4.6 × 4.6	75
.01	4.1 × 4.1	53
.1	3.3 × 3.3	28
.2	3.3 × 3.3	28
.3	2.8 × 2.8	17
.4	2.8 × 2.8	17
.5	2.8 × 2.8	17
.6	2.6 × 2.6	14
.7	2.6 × 2.6	14

TABLE 5*d*—EFFECT OF NaCl ON SIZE OF YEAST CELLS (*S. ellipsoides*)

Concentration of salt (M.NaCl)	Av. length and breadth of of yeast cells in Mu.	Av. volume of yeast cells calculated from length and breadth
.00	5.1 × 4.8	91
.001	5.0 × 4.4	75
.01	4.4 × 3.3	37
.1	4.1 × 3.3	34
.2	2.6 × 2.6	14

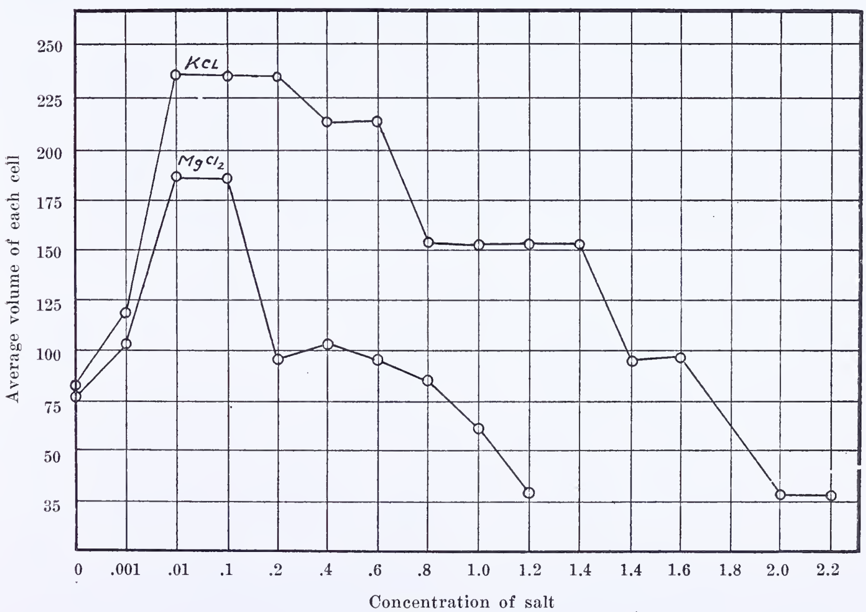

Fig. 5*a*.—Curves showing the average relative volumes of yeast cells in various concentrations of $CaCl_2$ and $MgCl_2$. The ordinates represent the average volume of the yeast cells and the abscissae, the concentrations of KCl and $MgCl_2$ used. The ordinate at 0 represents the volume in blank cultures.

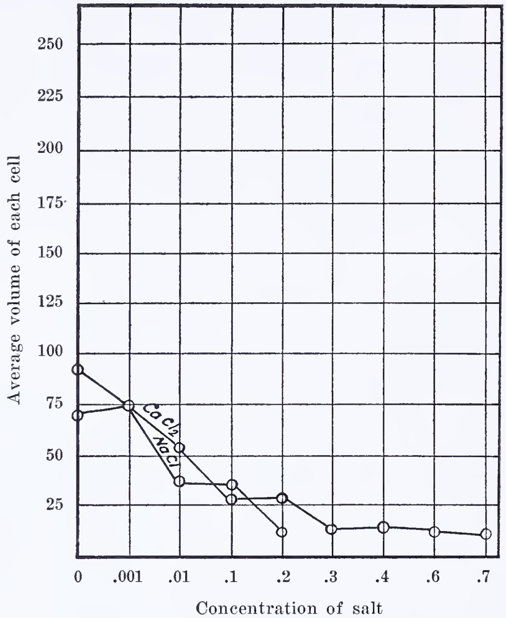

Fig. 5*b*.—Curves showing the average relative sizes in volumes of yeast cells in various concentrations of KCl and $MgCl_2$. The ordinates represent the volume of the yeast cells and the abscissae, the concentrations of the salts used. The ordinate at 0 represents the volume in blank cultures.

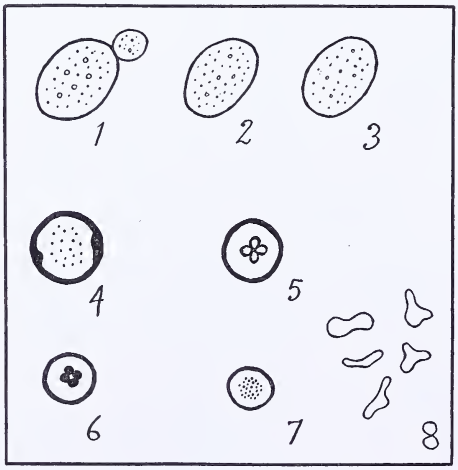

Fig. 5*c*.—Appearance of yeast cells in extreme concentrations of salts.

Normal yeast cells in 1, 2 and 3, diseased yeast cells from extreme concentrations of KCl and $MgCl_2$ in 4 and 5; (white) and diseased yeast cells from extreme concentrations of $CaCl_2$ and NaCl in 6, 7 and 8 (black or shadowy) (X5000).

The measurements given are the average of five counts in each case. The volumes from which the curves have been drawn in figure 5 (*a, b*) have been calculated, for purposes of comparison, as though the cells were cylindrical.

Both from table 5 (*a, b, c, d*) and the curves in figure 5 (*a, b*) it is evident that KCl and $MgCl_2$ favor growth in size up to the most favorable concentration, beyond which the cells decrease in size until the extreme concentration is reached, where they dissolve. Both NaCl and $CaCl_2$ limit the growth even in minute concentrations, thus showing their extreme toxicity to yeast cells.

Yeast cells seem to have remarkable resistant power. Many of them with cell wall thickened to a heavy membrane have been found in extreme concentrations. Perhaps this heavy membrane is formed to resist the osmotic pressure outside the cell. Besides some of the cells in these extreme concentrations are in normal condition and are even budding, thus showing the power of adaptability of yeast cells to new conditions. After they have become habituated to the presence of toxic salts, they grow normally and reproduce. It is probably owing to the adaptability of yeast to different conditions that the same yeast, *S. ellipsoideus,* collected from various sources, shows dissimilar physiological characters. Besides in many cases I have observed that the diseased yeast cells in extreme toxicity of KCl and $MgCl_2$ form a white membrane with normal cell contents, while those of $CaCl_2$ and NaCl form a rather dark cell membrane with shadowy cell contents. A similar case to that of Loeb[5, 13] may be cited here. In his experiments with sea urchin eggs he found two distinct phases of cytolysis which he terms "black cytolysis" and "white cytolysis."

With regard to the effect of the salts on the size of the yeast cells, NaCl is the most and KCl the least toxic, while $CaCl_2$ and $MgCl_2$ stand midway. The effect is parallel with that of the multiplication of cells.

An experiment was carried on with a second culture of *S. ellipsoideus* collected from another source by Cruess and named no. 60. This experiment was also made in duplicate. With this yeast potassium chloride and magnesium chloride gave the same results as with no. 66, but NaCl and $CaCl_2$ showed a marked difference and $CaCl_2$ was the most toxic of all. 4M NaCl gave an appreciable number of yeast cells, while even .3M $CaCl_2$ stopped the growth altogether. Further, the number of yeast cells was much lower than that of yeast no. 66. Evidently yeast no. 60 was less vigorous than the other, though otherwise there was no fundamental difference between them.

B. Experiments With Combinations of Salts—Antagonistic Effects

The toxic effects of the single salts KCl, $MgCl_2$, $CaCl_2$ and NaCl upon a wine yeast, *S. ellipsoideus,* have been shown in the first part of this paper. The results of the study indicate that the reactions of yeast differ from those of plants, animals or bacteria. This second part of the paper gives the results of an investigation to ascertain the effects of various binary combinations of the salts named upon the same yeast.

From the four salts, six combinations of two salts each are possible. All of these were tested. Judging from analogous work of other investigators with animals, plants and bacteria, it was expected that these salts would exhibit mutually antagonistic action, i.e., that the toxicity of one salt would be reduced by the presence of another and that the total effect of two salts together would be less than the sum of their individual effects. In some cases definite antagonistic effects were found. In others antagonism was not so well defined. In a few instances there was no antagonism shown.

In the discussion of results, considerable space has been given to the findings of other investigators because it was considered important to point out how the effects on other organisms compare with those on yeast. A few words on the development of the idea of antagonism in binary combinations of salts will be of value as an introduction to the data in this paper.

Considerable work on the antagonistic effects of salts has been done by Ringer, Locke, Howell, Loeb, Osterhout, Overton, Ostwald, Loew, Lipman and others. That the poisonous effect of one salt is reduced by the addition of another salt has been known for a long time, especially among animal physiologists. In this matter we owe a great deal of our knowledge to Loeb, whose investigations brought forth a large number of unexpected results. It was he who first developed the theory that the valences of metallic ions have considerable influence on their toxic and antagonistic effects, and that monovalent cations may be antagonized by bivalent, trivalent or tetravalent but not by monovalent cations. His results show some parallelism to the work of Linder and Picton.* This general statement does not apply in all cases to plants, animals and bacteria, experimented upon by various other investigators. Neither does it apply always to yeast.

* Hober and Gordon, Beitr. zur chem. physiol., vol. 5, p. 432, 1904, cited by Osterhout.[22]

The experiments with binary salts were made in the same general way as those with simple salts, but with slight modifications of technique. The flasks were arranged as before in duplicate, but in combining the salts in different molecular concentrations the method followed differed from those of previous investigators.

Of the two salts to be tested for antagonism, one was weighed from the minimum concentration to that of extreme toxicity according to the molecular concentration, and the other was weighed and added to the former in the reverse way in the corresponding flasks. The flasks containing the extreme concentration of each salt did not receive any addition of the other salt. Aside from this, the methods of inoculation, incubation, and microscopical counting were the same as those described for the single salts. Duplicates were made in all cases and two blanks were used in each series, as checks on the growth of the yeast in the treated flasks. The same yeast, *S. ellipsoideus*, no. 66, was employed in these experiments as in the ones with simple salts. The results given are therefore the average of duplicate experiments.

SERIES VI—ANTAGONISM BETWEEN MAGNESIUM CHLORIDE AND CALCIUM CHLORIDE

In this series $MgCl_2$ and $CaCl_2$ were combined in various molecular concentrations. A series of 16 Erlenmyer flasks was arranged in duplicate with two blank cultures. First, amounts of $MgCl_2$ corresponding to from 0M to 2.2M were weighed and put in the flasks, as was done with the single salts. The $CaCl_2$ also was weighed according to its molecular concentration and put in the same flasks in reverse order, leaving the extreme concentrations of each salt free from the addition of the other. Thus the first two flasks received .72M $CaCl_2$ without any addition of $MgCl_2$; the second received .66M $CaCl_2$ and .001 $MgCl_2$; the third .60M $CaCl_2$ and .01M $MgCl_2$, and so on to the last couple, which contained only 1.2M $MgCl_2$ and no addition of $CaCl_2$. The remaining flasks were combined in different molecular concentrations, as shown in table 1. Two blanks were taken to which no salt was added.

In order to facilitate the plotting of the curves, the different combinations of salts have been indicated by letters A, B, C, D, etc. *A* represents the blank cultures, while the other letters represent the different molecular combinations shown in the table below:

TABLE 6—ANTAGONISTIC EFFECT BETWEEN $MgCl_2$ AND $CaCl_2$

No.	$MgCl_2$ vs. $CaCl_2$ M. Conc.	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00 × .00	1,954,000	6,290,000	10,556,000	14,108,000	16,944,000
B	.00 × .72	....	....	....	....	....
C	.001 × .66	....	....	....	226,000	452,000
D	.01 × .60	452,000	2,034.000	3,842,000	4,520,000	5,650,000
E	.1 × .48	8,362,000	20,860,000	23,120,000	24,730,000	26,842,000
F	.2 × .36	10,848,000	26,024,000	29,706,000	30,856,000	31,960,000
G	.4 × .18	9,718,000	28,996,000	32,284,000	33,974,000	36,120,000
H	.6 × .06	8,804,000	25,286,000	30,256,000	31,865,000	32,556,000
I	.8 × .01	452,000	4,972,000	8,289,000	10,298,000	12,684,000
J	1.0 × .001	....	226,000	1,130,000	2,260,000	3,129,000
K	1.2 × .00	....	....	....	....	....

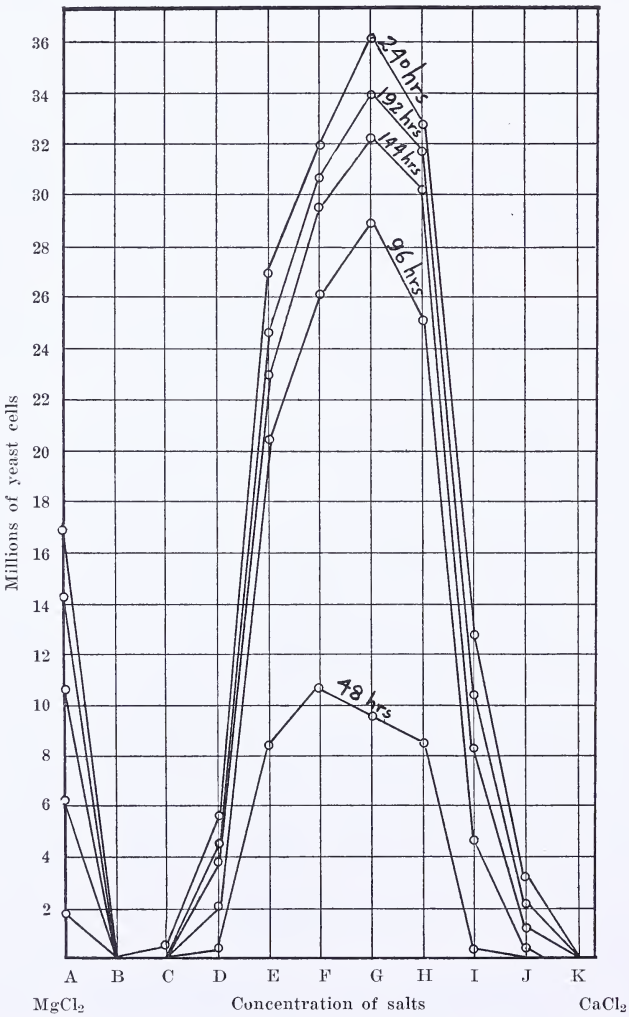

Fig. 6.—Curves of yeast growth showing antagonism between $MgCl_2$ and $Cacl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of the salts in combination. The ordinates at A represent the number of yeast cells in blank cultures.

From both table 6 and the curves in figure 6 it is evident that there is a distinct antagonism between these two salts. For example, in the experiments with simple salts $MgCl_2$ alone at .8M concentration allowed the growth of yeast cells up to only 8½ millions, but in combination with .01M $CaCl_2$ the growth was increased up to 12½ millions, i.e., 50 per cent increase. Similarly, .6M $CaCl_2$ alone allowed an increase to about one millions, and, with the addition of .01 $MgCl_2$, an increase to 5½ millions, showing 5½ times more growth. The highest number in $MgCl_2$ alone was 26½ millions at .1M, and in $CaCl_2$ alone 19 millions at .01M concentration. In this binary combination the highest number was obtained at G, the point where .4M $MgCl_2$ and .18M $CaCl_2$ were combined with a ratio of about 2:1.

For purposes of comparison let us now consider the results obtained in similar experiments with these four salts on plants, animals, and bacteria.

(*a*) *Plants.*—Kearney and Cameron[8] found a distinct antagonism between Mg and Ca ions for higher plants. In their experiments with leguminous plants *Lupinus albus* and *Medicago sativa* they found that, for a combination of these two salts, the plants show about five times as much tolerance as for the salts separately. The plants also displayed a remarkable degree of tolerance when $MgSO_4$ was used instead of $MgCl_2$, thus showing in addition the relative difference between different anions of the same salt.

Loew and his pupils,[10, 18] in their experiments with lower plants (*Spirogyra*), have found a strong antagonism between Mg and Ca ions.

(*b*) *Animals.*—Loeb[2] with sea urchins (blastulae and gastrulae) found that a mixture of $MgCl_2$ (10/8n) and $CaCl_2$ (10/8n) will allow them to swim for about forty-eight hours, while each of the salts singly at the same concentration is extremely poisonous and kills the animals. The same investigator[15] working with a jellyfish (*Polyorchis*) has shown that the addition of a small quantity of $CaCl_2$ to a mixture of NaCl and $MgCl_2$ favors the normal, rhythmical contractions, while $MgCl_2$ alone stops them altogether. Contrary to the above results, Loeb[12] in his experiments with frogs has found that a combination of Mg and Ca ions completely inhibits the rhythmical muscular contractions. This has been corroborated by Anne Moore,[7] in her experiments with the contraction of the lymph hearts of frogs.

Lillie[6] has found that the ciliary movement of the larvae of

Arenicola goes on normally for a time in a mixture of $MgCl_2$ and $CaCl_2$ with the ratio of 4:1 at 10/8n concentration, though either of the two salts used alone would stop it entirely. Matthews,[11] in his work with the development of embryos in the eggs of *Fundulus,* found a distinct antagonism between Mg and Ca.

Meltzer and Auer[21] have shown with rabbits and a monkey that the poisonous action of $MgCl_2$ in subcutaneous injection is similarly diminished by the injection of $CaCl_2$. They found also a strong antagonism between the nitrates, acetates and sulfates of these two salts respectively.

(*c*) *Bacteria.*—Lipman,[23, 24] with a soil bacterium, *Bacillus subtilis,* found little or no antagonism between the two salts, but, on the contrary, the addition of one salt to the other was found to be more toxic than either of the two salts used alone.

All of the above mentioned experiments, except those of the three cases of Lipman, Loeb, and Anne Moore, are in agreement with the antagonistic effects between Mg and Ca ions that occur with yeast. In addition, it may be noted here that the antagonistic effect between $MgCl_2$ and $CaCl_2$ with yeast has been found to be the strongest of all the combinations. This corroborates the opinion advanced by Loew that there is a strong antagonism between calcium and magnesium both with plants and animals.[10]

SERIES VII—ANTAGONISM BETWEEN POTASSIUM CHLORIDE AND CALCIUM CHLORIDE

In this series the experiments were carried on in the same way as with $MgCl_2$ and $CaCl_2$. Table 7 and the curves in figure 7 show there is a distinct antagonism between the two salts. In this case marked antagonism was found on the side of $CaCl_2$, but little or none on the side of KCl. For example, the combination of .001M KCl with .66M $CaCl_2$ allowed the yeast to grow up to 6½ millions, while in $CaCl_2$ at .6 alone the yeast was found to increase only up to about one million. Thus there was 6½ times as much growth where the KCl was present. But, on the other hand, the combination of .001M $CaCl_2$ to 2.0M KCl did not accelerate the growth. This unexpected result may be accounted for by the fact that the higher concentrations of KCl being very high in comparison to the small concentrations of $CaCl_2$ the latter was not sufficient to reduce the toxicity of the KCl at such a high concentration. It is also very probable that a concentration of 2.0M KCl exerts a strong osmotic effect and that the toxicity is due to osmotic influences rather than to the usual toxicity of the ion itself. If this were true we would expect little antagonism from other salts.

Loeb[12] in his experiments with a jellyfish (*Gonionemus*) met with a similar difficulty. In this case the KCl concentration was so high that a small concentration of NaCl did not remove the toxicity, and so the combination inhibited the contraction of the animal, while the same concentrations used in the case of another kind of fish, *Fundulus,* allowed the development of embryos in the eggs. He has pointed out the fact that in the embryos of *Fundulus* the solutions in which cleavage proceeds normally interferes seriously with the heartbeat of *Gonionemus,* if the proportion of KCl exceeds a certain limit. In this instance we find proof of the fact that in the same organism cell-division and muscular contractility are influenced by entirely different combinations of ions, and therefore these vital activities must depend on widely different chemical constitutions. However, the highest growth in the case of yeast was obtained at H, where .6M KCl and .36M $CaCl_2$ have been combined, a ratio of about 2:1. In the case of KCl alone the highest growth was obtained at .2M concentration, allowing growth up to 30½ millions per c.c. $CaCl_2$ allowed growth up to 19 millions at .01M concentration.

TABLE 7—ANTAGONISTIC EFFECTS BETWEEN KCL AND $CACL_2$

No.	KCl vs. $CaCl_2$ M. Conc.	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00 × .00	2,101,000	8,589,000	13,908,000	16,896,000	17,520,000
B	.00 × .72	....	....	....	....	....
C	.001 × .66	....	226,000	2,034,000	3,985,000	6,722,000
D	.01 × .60	452,000	4,972,000	13,315,000	18,604,000	22,720,000
E	.1 × .54	4,020,000	10,328,000	20,245,000	23,266,000	25,120,000
F	.2 × .48	5,558,000	12,840,000	22,190,000	26,880,000	29,380,000
G	.4 × .42	3,034,000	12,840,000	26,852,000	19,126,000	32,285,000
H	.6 × .36	1,017,000	8,398,000	13,645,000	28,904,000	34,500,000
I	.8 × .30	226,000	4,256,000	10,250,000	14,966,000	18,732,000
J	1.0 × .24	....	2,965,000	6,126,000	9,551,000	16,159,000
K	1.2 × .18	....	1,130,000	3,986,000	5,410,000	7,119,000
L	1.4 × .12	....	452,000	2,550,000	2,712,000	4,438,000
M	1.6 × .06	....	....	904,000	1,130,000	3,906,000
N	1.8 × .01	....	....	452,000	904,000	2,652,000
O	2.0 × .001	....	....	....	226,000	1,130,000
P	2.2 × .00	....	....	....	....	....

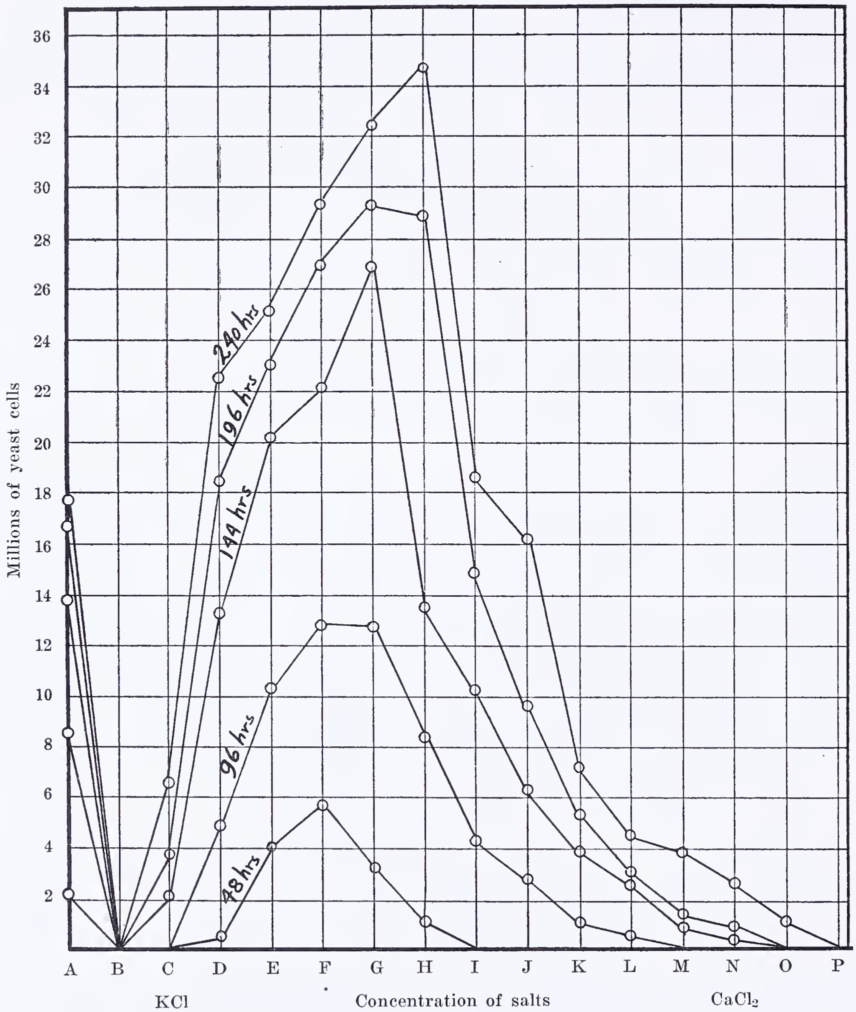

Fig. 7.—Curves of yeast growth showing antagonism between KCl and $CaCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at A represents the number of yeast cells in blank cultures.

For comparison with these results, a number of cases dealing with plants, animals and bacteria may be cited below:

(*a*) *Plants.*—Osterhout[22] has shown that for higher plants a combination of 100 c.c. KCl and 5 c.c. $CaCl_2$ at the concentration of .12M is best suited for the highest development of roots. Benecke[19] has shown that for lower plants (*Spirogyra*) the harmful effect of the K ion is very distinctly antagonized by the addition of the Ca ion at a certain definite concentration.

(*b*) *Animals.*—In regard to the development of embryos in the eggs of *Fundulus,* Loeb[1] has met with a marked antagonism between the two salts, using 75 c.c. of KCl (5/8n) and 25 c.c. of $CaCl_2$ (10/8n). This combination allowed the development of a number of embryos, while in the same concentration of KCl alone no development was shown. He also obtained a similar result with the muscular contraction of a jellyfish (*Polyorchis*),[15] thus showing an antagonistic effect between the two salts. The same investigator[3] in his experiments with the hydromedusa *Gonionemus* has shown that the combination of K ion (5/8n) and Ca ion (10/8n) is poisonous to the animals. Anne Moore[7] obtained a similar result in her experiments on the contraction of the lymph heart of frogs.

Meltzer and Auer[21] have shown that with rabbits and a monkey in subcutaneous injection there is a limited antagonism between the two salts. Matthews[11] with *Fundulus* met with a similar result. He found that at the dilution of M/1600 $CaCl_2$ to 6/8n KCl the development of embryos in the eggs was found to be the best.

Lillie[6] found that with the larvae of *Arenicola* the ciliary activity went on when he used 97.5 c.c. $CaCl_2$ (10/8n) and 2.5 c.c. KCl (5/8n), showing an antagonism between the two salts.

(*c*) *Bacteria.*—Lipman[23] has shown that for *Bacillus subtilis* the highest production of ammonia is found at the point where 100 c.c. KCl and 5 c.c. $CaCl_2$ at the concentration of .35M is used, thus showing a distinct antagonism between the two salts. His work has a striking similarity to that of Osterhout on wheat.

Summarizing the antagonism between K and Ca, it may be said that the toxicity of high concentrations of Ca is greatly reduced by the presence of K, but that the toxicity of high concentrations of K is not appreciably reduced by small amounts of Ca. The optimum ratio of KCl to $CaCl_2$ was about 2:1 for yeast.

SERIES VIII—ANTAGONISM BETWEEN MAGNESIUM CHLORIDE AND SODIUM CHLORIDE

The experiments in this series were carried on in the same way as the others. Both table 8 and the curves in figure 8 show that there is a distinct antagonism between these two salts. The highest growth in this case was found at G, the point where .4M $MgCl_2$ and .06M NaCl were combined, a ratio of about 6:1. As already shown, when used singly $MgCl_2$ allows the highest growth at .1M, i.e., 26½ millions,

TABLE 8—ANTAGONISTIC EFFECTS BETWEEN $MgCl_2$ AND NaCl

No.	$MgCl_2$ vs. NaCl M. Conc	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00 × .00	3,100,000	7,644,000	13,686,000	15,203,000	17,009,000
B	.00 × .208	....	....	....	....	....
C	.001 × .180	....	....	....	226,000	226,000
D	.01 × .160	226,000	452,000	452,000	678,000	1,130,000
E	.1 × .128	3,250,000	6,212,000	11,201,000	14,258,000	17,255,000
F	.2 × .096	5,424,000	10,396,000	16,368,000	21,690,000	25,793,000
G	.4 × .064	2,260,000	12,656,000	20,696,000	25,882,000	28,890,000
H	.6 × .032	1,582,000	8,684,000	14,956,000	17,009,000	21,583,000
I	.8 × .01	904,000	3,102,000	6,358,000	10,605,000	15,430,000
J	1.0 × .001	....	904,000	1,872,000	3,896,000	4,276,000
K	1.2 × .00	....	....	....	....	....

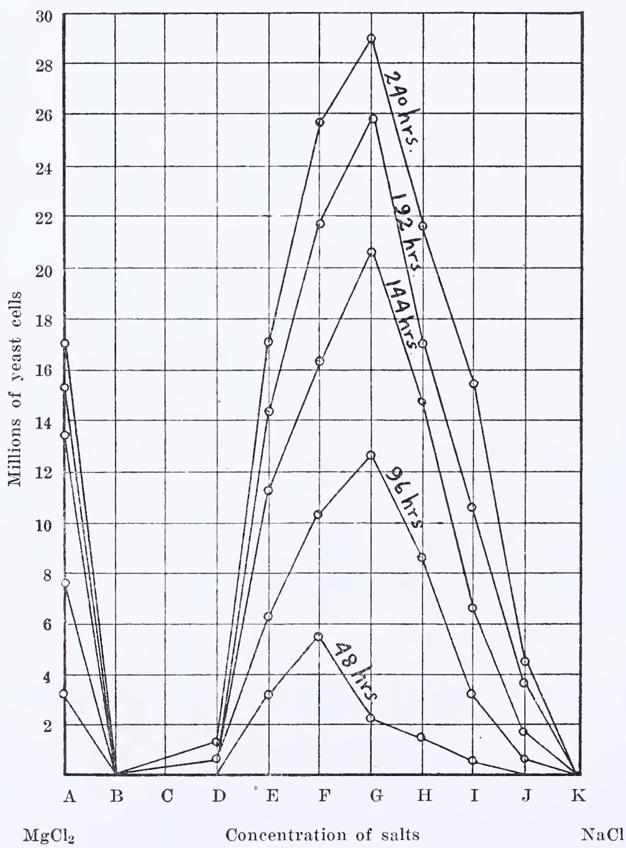

Fig. 8.—Curves of yeast growth showing antagonism between $MgCl_2$ and NaCl. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at A represents the number of yeast cells in blank cultures.

and NaCl at .001M, i.e., 18 millions. But in combination the two salts permit the highest growth of 29 millions per c.c. at .4M and .06M respectively.

The antagonism between these two salts in the case of yeast is found very distinctly at both ends of the curves. For example, .1M NaCl alone shows a growth of scarcely more than one million, while in combination with .1M $MgCl_2$ it shows over 17 millions, or 17 times as much. On the other hand, .8M $MgCl_2$ alone allowed a growth of about 8½ millions, while in combination with .01M NaCl the growth was increased to about 15½ millions, or about twice as much.

In comparison with these results, a number of cases dealing with the effects of combinations of $MgCl_2$ and NaCl on plants, animals and bacteria are cited below.

(*a*) *Plants.*—Osterhout[5] found a distinct antagonism between the two salts with the growth of a fungus (*Botrytis cinerea*). He found that 15.M NaCl alone was very toxic, but that when this concentration of NaCl was combined with .4 M $MgCl_2$ the toxicity was much reduced. He also found with wheat that neither NaCl nor $MgCl_2$ at .12M alone allowed root development, but in a combination in the proportion of 100 c.c. NaCl to 75 c.c. $MgCl_2$ the root developed very well. The same investigator obtained a negative result with green algae.[20]

Kearney and Cameron[8] with *Lupinus albus* and *Medicago sativa* have shown that the addition of $MgCl_2$ to NaCl raised the tolerance of these plants to the latter 3–10 times.

(*b*) *Animals.*—Loeb[12] with *Fundulus* has found that in a mixture of 98 c.c. 5/8n NaCl and 2 c.c. 10/8n $MgCl_2$ all the eggs develop embryos, while the same salts alone at the same concentration are extremely toxic. Even an equal proportion of the two salts in the same concentration allowed about 75 per cent of the embryos to develop. He also found a similar antagonism with a sea urchin (*Arbacia*) and a jellyfish (*Polyorchis*).

Lillie[6] found that with the larvae of *Arenicola* the ciliary movement continued for a time when he added 10 c.c. $MgCl_2$ (10/8n) to 90 c.c. NaCl (5/8n), while the same concentrations of NaCl alone would stop it immediately. Matthews with *Fundulus* found an antagonism between the two salts.

Ostwald,[13] however, with fresh-water *Grammarus* obtained contrary results. In this case a combination of the two salts was found to be more toxic than NaCl alone, isotonic with sea water (2.7 per cent NaCl in sea water or about .4M NaCl).

(*c*) *Bacteria.*—Lipman[23] with *Bacillus subtilis* obtained a result similar to that of Osterhout. A mixture of the same concentration of $MgCl_2$ and NaCl (.35M) in the ratios of 10:1 gave the maximum production of ammonia.

To summarize the results of these experiments, it may be said that there is a distinct antagonism between $MgCl_2$ and NaCl, which is evident on both ends of the curves in figure 8. In this case the yeast agrees with the observations on plants, animals and bacteria except in the two instances cited above in regard to fresh-water *Grammarus* and green algae.

SERIES IX—ANTAGONISM BETWEEN POTASSIUM CHLORIDE AND SODIUM CHLORIDE

In this series the flasks were arranged as before. It has been pointed out by Loeb that two salts with ions of like valence, especially in the case of monovalent ions, do not antagonize the toxicity of each other, but rather show a moderately increased toxicity when combined. This is evident with yeast, as is shown by table 9 and the curves in figure 9. The highest growth in this case was found at F, where .2M KCl and .12M NaCl have been combined, having a ratio of about 2:1. KCl alone at .2M concentration allows the growth about 1½ times that found in this combination. Thus the antagonism of NaCl for KCl is found to be negative. But, on the other hand, there is a distinct antagonism of KCl for NaCl. For example, NaCl alone at .17M concentration hardly allowed any growth, but in combination with .01M KCl the growth was accelerated up to about 15 millions, thus showing a distinct antagonism. The reason of this unexpected negative result on the side of KCl is perhaps the same that I have suggested in the case of KCl and $CaCl_2$ in Series II.

For comparison with these results, a number of cases dealing with plants, animals and bacteria are cited below:

(*a*) *Plants.*—Osterhout[22] with wheat (Early Genésee) has found a slight antagonism between K and Na ions. But in his work[17] on a green alga he obtained a negative result using 3/8M concentration of two salts in combination.

(*b*) *Animals.*—Loeb[1] with *Fundulus* found a slight antagonism between the K and the Na ion in relation to the development of em-

TABLE 9—ANTAGONISTIC EFFECTS BETWEEN KCL AND NACL

No.	KCl vs. NaCl	M. Conc	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00	× .00	1,954,000	6,290,000	10,556,000	14,108,000	16,944,000
B	.00	× .208	....	....	....	....	....
C	.001	× .192	....	226,000	1,808,000	6,780,000	7,888,000
D	.01	× .176	2,678,000	7,184,000	9,256,000	12,176,000	14,952,000
E	.1	× .160	5,424,000	10,786,000	14,690,000	16,922,000	18,566,000
F	.2	× .144	2,938,000	12,339,000	15,942,000	18,206,000	21,250,000
G	.4	× .128	2,260,000	7,838,000	11,526,000	15,830,000	17,248,000
H	.6	× .112	678,000	6,780,000	8,678,000	12,687,000	13,266,000
I	.8	× .096	226,000	5,650,000	7,205,000	10,256,000	12,984,000
J	1.0	× .080	....	4,156,000	5,882,000	8,120,000	9,886,000
K	1.2	× .064	....	2,906,000	4,900,000	7,750,000	8,205,000
L	1.4	× .048	....	1,130,000	3,390,000	4,968,000	6,983,000
M	1.6	× .032	....	452,000	1,130,000	2,260,000	4,452,000
N	1.8	× .010	....	....	226,000	1,130,000	2,960,000
O	2.0	× .001	....	....	....	452,000	904,000
P	2.2	× .00	....	....	....	....	....

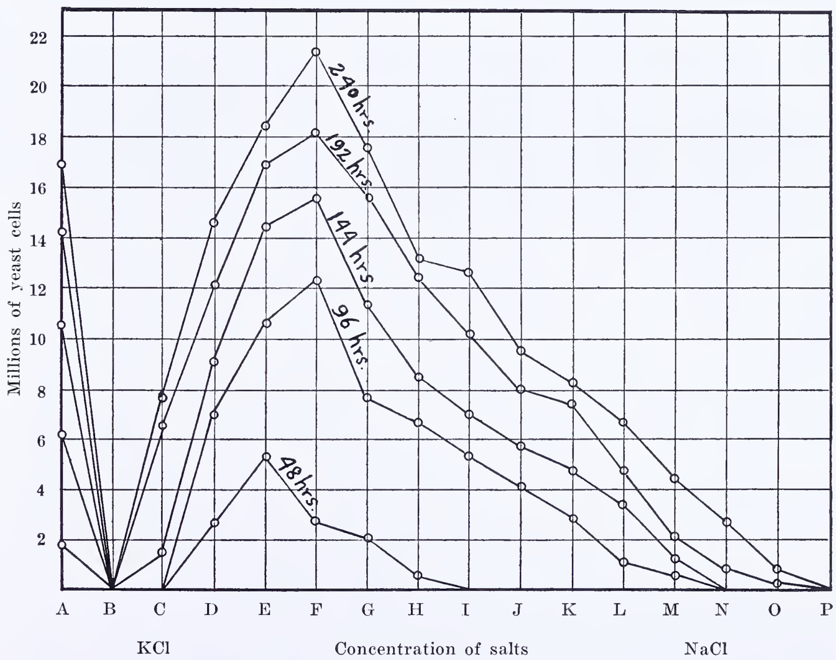

Fig. 9.—Curves of yeast growth showing antagonism between KCl and Nacl. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at A represents the number of cells in blank cultures.

bryos in the eggs. He also found a similar result with sea-urchins, *Hydromedusa gonionemus,* and a jellyfish, *Polyorchis.*

Lillie[6] found that with the larvae of *Arenicola* the ciliary movement goes on in a solution containing 20 parts of NaCl (5/8n) and 8 parts of KCl (5/8n), while each salt used alone stops the movement altogether.

Ostwald[13] with fresh-water *Gammarus* has shown that there is a distinct antagonism between K and Na ions in regard to the duration of life of that animal. Matthews[11] has found that it takes twice as much KCl to neutralize the toxicity of NaCl in the case of the development of embryos in the eggs of *Fundulus.* This is rather similar to the case of yeast, where it takes .2M KCl to neutralize the toxicity of .14M NaCl to allow the highest growth.

(*c*) *Bacteria.*—Lipman[23] with *Bacillus subtilis* has found that none of the combinations of these two salts gives as favorable conditions for growth as is found with each salt alone at the same concentration, thus showing non-antagonism between the two salts.

To summarize the results in this experiment, it may be said that with yeast, like valences prevent the antagonistic effects, contrary to what was found by Lipman with soil bacteria, but in accordance with the results of Osterhout with wheat, Loeb with *Fundulus,* and other investigators with other organisms. The yeast agrees in this case with all the above-mentioned cases except with that of green algae tested by Osterhout and that of *Bacillus subtilis* by Lipman.

SERIES X—ANTAGONISM BETWEEN POTASSIUM CHLORIDE AND MAGNESIUM CHLORIDE

The experiments in this series were conducted like the others. The highest growth in this case was found at H, the point where .6M KCl and .5M $MgCl_2$ were combined in a ratio of about 1:1. In the case of simple salts KCl alone at .2M concentration allowed the highest growth up to about 30½ millions and $MgCl_2$ at .1M about 26½ millions. KCl alone at .6M and $MgCl_2$ at .5M permitted the growth of yeast more than is found in this combination at H. But this indicates a mild antagonism, because the toxic effect was less than the sum of the separate toxic effects of the two salts used alone. Distinct antagonism to the effects of $MgCl_2$ is shown by KCl, but not the converse. For example, .8M $MgCl_2$ alone allows the yeast to grow only to 8 millions, while in the combination with .1M KCl the growth has been increased to 11½ millions. On the other hand, the smaller concentrations of $MgCl_2$ with higher concentrations of KCl did not show any antagonism. The reason for this unexpected result is perhaps that previously mentioned in the case of KCl vs. $CaCl_2$ in Series VII.

Table 10—Antagonistic Effects Between KCl and $MgCl_2$

No.	KCl vs. $MgCl_2$ M. Conc.	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00 × .00	2,356,000	9,701,000	12,170,000	14,890,000	17,526,000
B	.00 × 1.2	....	....	....	....	....
C	.001 × 1.0	....	226,000	1,130,000	2,260,000	3,845,000
D	.01 × .9	226,000	1,356,000	6,780,000	10,070,000	11,560,000
E	.1 × .8	1,130,000	6,780,000	7,408,000	10,975,000	12,180,000
F	.2 × .7	1,356,000	7,458,000	11,578,000	13,449,000	14,328,000
G	.4 × .6	2,486,000	4,838,000	7,006,000	14,690,000	15,500,000
H	.6 × .5	904,000	3,816,000	5,296,000	12,850,000	16,280,000
I	.8 × .4	678,000	2,612,000	4,852,000	8,286,000	9,856,000
J	1.0 × .3	452,000	2,260,000	3,706,000	5,463,000	5,902,000
K	1.2 × .2	452,000	2,040,000	3,295,000	4,895,000	5,240,000
L	1.4 × .1	678,000	1,926,000	2,940,000	3,656,000	4,864,000
M	1.6 × .05	226,000	904,000	3,050,000	3,006,000	4,628,000
N	1.8 × .01	....	226,000	2,260,000	2,990,000	3,862,000
O	2.0 × .001	....	....	....	2,226,000	1,130,000
P	2.2 × .00	....	....	....	....	....

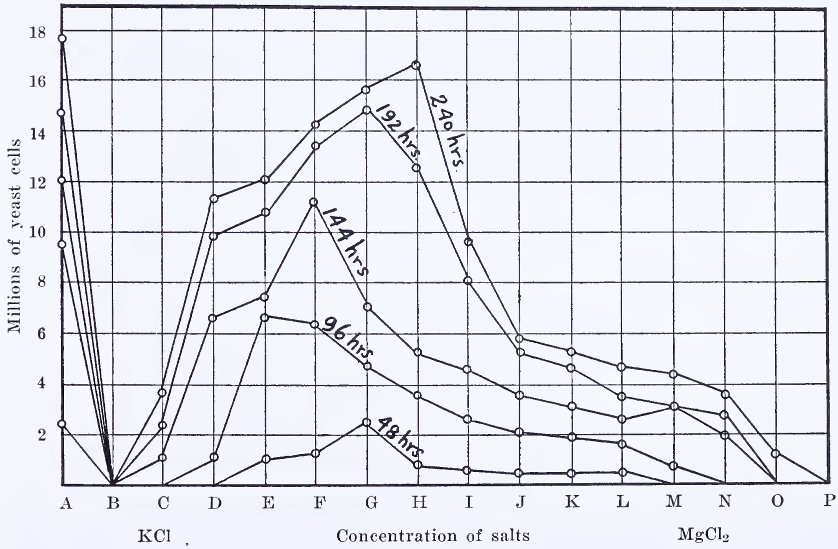

Fig. 10.—Curves of yeast growth showing antagonism between KCl and $MgCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at A represents the number of cells in blank cultures.

For comparison with these results a few cases may be cited as follows:

(*a*) *Plants.*—Osterhout[20] with wheat (Early Genésee) has shown that the root develops better in a solution having 100 c.c. KCl and 7.5 c.c. $MgCl_2$ at .12M concentration than in KCl alone. He also found with a marine alga,[20] *Enteromorpha hopkirkii,* that both salts are poisonous when used alone, but a combination in the proportion of 100 c.c. $MgCl_2$ and 40 c.c. KCl allows considerable growth. He found a similar antagonism with liverworts.[20]

(*b*) *Animals.*—Matthews[11] found with *Fundulus* that in order to permit development of the embryos in the eggs at the concentration of 33/48n KCl at least about M/160 $MgCl_2$ is needed. He also found that a solution of 6/8n KCl requires M/80 $MgCl_2$ to give the best result.

(*c*) *Bacteria.*—Lillie[6] has shown that a combination of 10/8n $MgCl_2$ and 5/8n KCl allows the ciliary activity of the larvae of *Arenicola,* which is stopped when one salt is used alone.

To summarize, it may be said that a distinct antagonism was found by Osterhout with higher and lower plants and by Matthews and Lillie with animals. With yeast a slight antagonism is found, which is shown on the curves in figure 10 on the side of $MgCl_2$.

SERIES XI—ANTAGONISM BETWEEN CALCIUM CHLORIDE AND SODIUM CHLORIDE

The plan of this series of experiments was the same of that of the others. In the case of simple salts both $CaCl_2$ and NaCl were found to be very toxic, and it may be owing to this extreme toxicity that the combinations of the two salts showed increased toxicity. Both from table 11 and the curves in figure 11 it is evident that this toxicity is very marked. The highest growth was found at E, where .1M $CaCl_2$ and .12M NaCl have been combined in the ratio of 1:1. But even here the number of yeast cells went up only to 8 millions, which is far below the highest growth obtained when the salts were used alone. However, $CaCl_2$ shows slight antagonism to the toxicity of NaCl, for example, .1M $NaCl_2$ alone allows the growth only to one million, while in combination with .1M CaCl it reached more than 8 millions. On the whole, however, both from the table and the curves it is evident that the combinations of the two salts are more toxic than the single salts.

TABLE 11—ANTAGONISTIC EFFECTS BETWEEN $CaCl_2$ AND NaCl

No.	$CaCl_2$ vs. NaCl	M. Conc	48 hrs.	96 hrs.	144 hrs.	192 hrs.	240 hrs.
A	.00	× .00	2,356,000	9,381,000	12,172,000	14,890,000	17,108,000
B	.00	× .208	....	....	....	....	....
C	.001	× .18	226,000	226,000	226,000	904,000	1,130,000
D	.01	× .16	226,000	1,582,000	3,390,000	4,682,000	5,842,000
E	.1	× .12	226,000	1,808,000	5,650,000	7,910,000	8,290,000
F	.2	× .09	....	1,356,000	3,482,000	4,520,000	7,042,000
G	.3	× .06	....	226,000	1,130,000	5,650,000	6,820,000
H	.4	× .03	....	....	226,000	3,390,000	4,526,000
I	.5	× .01	....	....	....	....	452,000
J	.6	× .001	....	....	....	....	....
K	.7	× .00	....	....	....	....	....

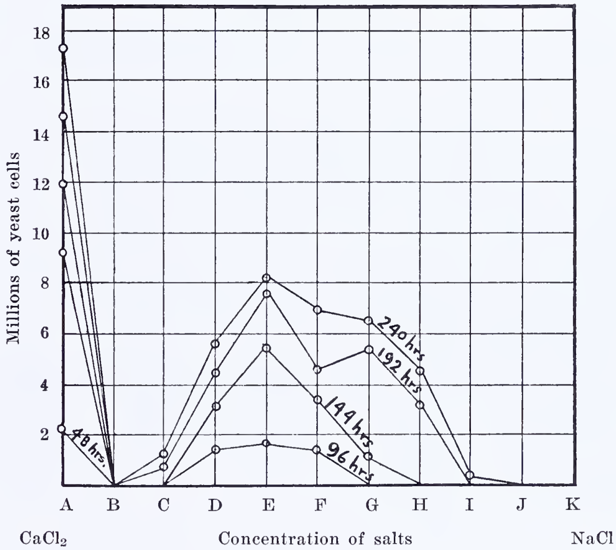

Fig. 11.—Curves of yeast growth showing effects of NaCl on $CaCl_2$. The ordinates represent the number of yeast cells in millions and the abscissae, the concentration of salts in combination. The ordinate at A represents the number of yeast cells in blank cultures.

For comparison with other organisms the following cases are cited:

(*a*) *Plants.*—Osterhout[5] with wheat found a distinct antagonism between the two salts. He obtained a similar result with green algae in which he used 100 c.c. NaCl and 10 c.c. $CaCl_2$ at the concentration of 3/8M. Kearney and Cameron,[8] with leguminous plants, found that a combination of the two salts increased the tolerance of the plants for $CaCl_2$ three times.

(*b*) *Animals.*—Loeb[1] with *Hydromedusa Gonionemus* has shown that a combination of 10/8n $CaCl_2$ and 5/8n NaCl is harmless to animals. He[15] also found a distinct antagonism with a jellyfish, *Polyorchis,* using 50 c.c. NaCl and 1 c.c. $CaCl_2$, which allowed the animal to swim, while NaCl alone was poisonous. The same investigator found a distinct antagonism between these two salts working with the development of embryos in the eggs of the *Fundulus.* Anne Moore[7] with the contraction of the lymph heart of frogs and Lingle[4] with that of the turtle's heart noted similar phenomena, thus corroborating the work of Loeb.

Lillie[6] working with the larvae of *Arenicola* has found a distinct antagonism between Ca and Na ions. MacCallum[14] found the same with his experiments on cathartics.

Meltzer and Auer[21] found a distinctly antagonistic effect with animals in subcutaneous injections. Ostwald[13] wth fresh-water *Grammarus* found a strong antagonism between NaCl and $CaCl_2$ in regard to the duration of life of that animal. Finally, Matthews[11] has shown that there is a slight antagonism between the two salts in the development of embryos in the eggs of *Fundulus.*

(*c*) *Bacteria.*—Lipman[24] with *Bacillus subtilis* found a marked lack of antagonism between the two salts. In his case any combination of the two salts at .35M concentration was found to be more poisonous than a single salt.

All these experiments except that of Lipman show that there is antagonism between $CaCl_2$ and NaCl. The yeast agrees very markedly with *Bacillus subtilis* in showing little or no antagonism between the two salts, $CaCl_2$ and $NaCl_2$.

Relative Antagonisms of Various Combinations

Table 12 is intended to show the relative antagonisms of the various combinations. The data used in constructing the table are the final counts in each flask.

The average of the counts in all the check flasks is taken as the basis from which to estimate the influence of the various salts and of their combinations. The calculation is made as follows:

Yeast growth in check flasks = 17 (millions).
Yeast growth with single salt no. 1 = a.
Yeast growth with single salt no. 2 = b.
Yeast growth with combination no. 1 + 2 = c.
Toxicity — expected = $(17 - a) + (17 - b)$.
Toxicity — observed = $17 - c$.
Antagonism of combinations* = $(17 - a) + (17 - b) - (17 - c)$.
$\therefore$ Antagonism = $17 + c - a - b$.

Table 12—Range of Antagonism of the Binary Combinations Calculated From the Last Microscopical Count*

No.	$MgCl_2 \times CaCl_2$	$KCl \times CaCl_2$	$MgCl_2 \times NaCl$	$KCl \times NaCl$	$KCl \times MgCl_2$	$CaCl_2 \times NaCl$
A†	17,000,000	17,000,000	17,000,000	17,000,000	17,000,000	17,000,000
B	....	....	....	....	....	....
C	....	4,000,000	....	5,000,000	4,000,000	....
D	4,000,000	17,000,000	....	8,000,000	10,000,000	4,000,000
E	27,000,000	17,000,000	8,000,000	9,000,000	9,000,000	7,000,000
F	24,000,000	18,000,000	25,000,000	8,000,000	7,000,000	7,000,000
G	20,000,000	30,000,000	27,000,000	7,000,000	16,000,000	6,000,000
H	21,000,000	31,000,000	14,000,000	7,000,000	9,000,000	....
I	7,000,000	8,000,000	11,000,000	10,000,000	....	....
J	1,000,000	1,000,000	2,000,000	10,000,000	....	....
K	....	....	....	1,000,000	....	....
L	....	....	....	....	....	....
M	....	....	....	....	....	....
N	....	....	....	....	....	....
O	....	....	....	....	....	....
P	....	....	....	....	....	....

† Millions on average.
* These results are shown graphically by the curves in figure 12.

* This defines 'antagonism' as the difference between the expected and the observed toxicity.

The curves have been drawn to show the antagonism of the combinations and not the actual growth of the yeast as has been shown in the previous curves.

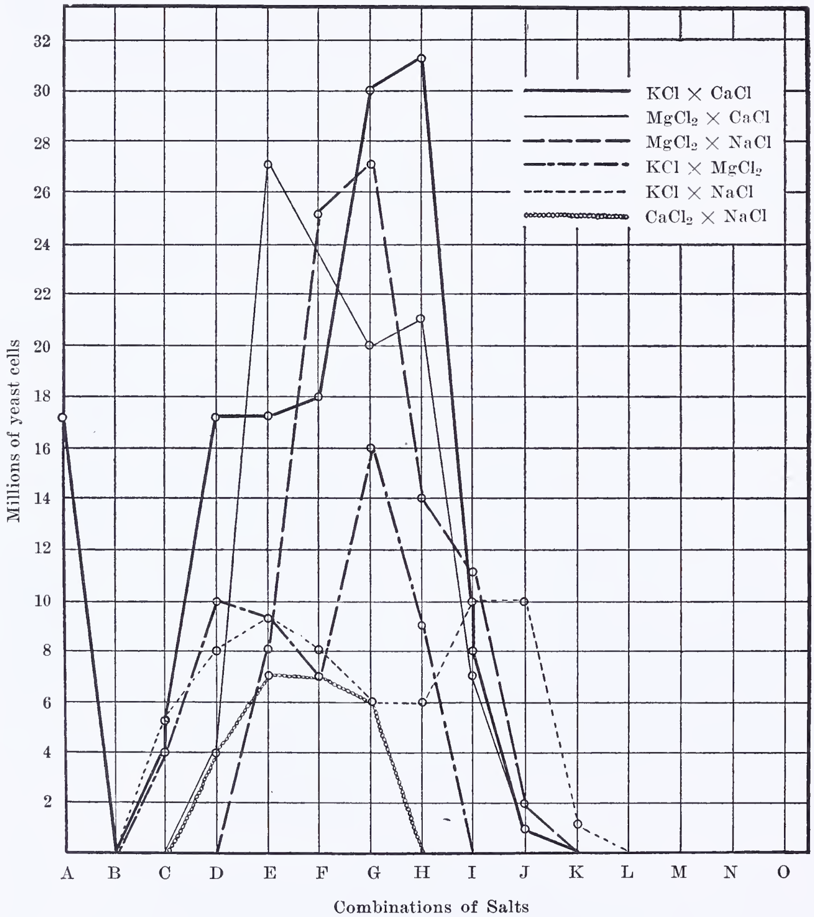

Fig. 12.—Curves showing range of antagonism of binary combinations of salts. The ordinates represent the average number of yeast cells in millions and the abscissae, the concentration of salts in combinations. The ordinate at A represents the average number of cells in blank cultures.

Summary

PART A—TOXIC EFFECTS OF SINGLE SALTS

1. Each of the four single salts—KCl, $MgCl_2$, $CaCl_2$, and NaCl—is more or less toxic to the yeast, *Saccharomyces ellipsoideus,* at certain concentration. KCl is the least toxic and NaCl the most for the yeast (no. 66) used.

2. The lower concentrations of each salt stimulate the growth of yeast. The highest number of yeast cells in microscopical count was found at .2M KCl, .1M $MgCl_2$, .01M $CaCl_2$, and .001M NaCl, KCl being the most favorable and NaCl the least. Beyond the favorable concentrations the various salts are toxic to yeast.

3. The concentrations of salts that inhibited the growth of yeast cells were found at 2.2M KCl, 1.2M $MgCl_2$, .7M $CaCl_2$, and .2M NaCl.

4. The results of the experiments are quite different from those found with either bacteria, the higher plants or animals. The yeast stands in this respect midway between plants and animals and swings to either direction according to the environment.

5. The salts used had a marked effect on the size and appearance of the yeast. Taking decrease in size as a criterion, the salts affected the yeast toxically in the same relative ways as indicated by the rate of multiplication of the cells.

PART B—ANTAGONISTIC EFFECTS OF COMBINATIONS OF SALTS

1. As shown by growth of yeast, the variation in antagonism between the four single salts in all possible combinations may be arranged in order as follows:

1. $MgCl_2$ vs. $CaCl_2$ (most)
2. KCl vs. $CaCl_2$
3. $MgCl_2$ vs. NaCl
4. KCl vs. NaCl
5. KCl vs. $MgCl_2$
6. $CaCl_2$ vs. NaCl (least)

2. The effect of binary salts with yeast, whether positively or negatively antagonistic in comparison to animals, plants and soil bacteria, may be tabulated as follows:

Binary salts	Yeast	Animals	Plants	Soil bacteria
1. $MgCl_2$ vs. $CaCl_2$	+	+ and –	+	—
2. KCl vs. $CaCl_2$	+ and –	+ and –	+	+
3. $MgCl_2$ vs. NaCl	+	+ and –	+ and –	+
4. KCl vs. NaCl	+ and –	+	+ and –	+ and –
5. KCl vs. $MgCl_2$	+ and –	+	+	....
6. $CaCl_2$ vs. NaCl	+ and —	+	+	—

+ = strong antagonism. + = mild antagonism. — = strong increase of toxicity. – = slight increase of toxicity.

3. In regard to the effects of valences of ions the following results have been obtained with yeast:

(*a*) That divalent ions may antagonize monovalent ions is evident from the combinations of $MgCl_2$ vs. NaCl and $CaCl_2$ vs. NaCl. Ngative results were obtained from the combinations of KCl vs. $CaCl_2$ and KCl vs. $MgCl_2$.

(*b*) That a divalent ion may be antagonized by a divalent ion is evident from the combination of $MgCl_2$ vs. $CaCl_2$.

(*c*) That monovalent ions may antagonize divalent ions is shown in the combinations of KCl vs. $CaCl_2$; $MgCl_2$ vs. NaCl and KCl vs. $MgCl_2$.

(*d*) That a monovalent ion may antagonize a monovalent ion, though not very markedly, has been found in the combination of KCl vs. NaCl.

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